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Characterization of Factors Affecting Nanoparticle Tracking Analysis Results With Synthetic and Protein Nanoparticles

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ABSTRACT

In many manufacturing and research areas, the ability to accurately monitor and characterize nanoparticles is becoming increasingly important. Nanoparticle tracking analysis is rapidly becoming a standard method for this characterization, yet several key factors in data acquisition and analysis may affect results. Nanoparticle tracking analysis is prone to user input and bias on account of a high number of parameters available, contains a limited analysis volume, and individual sample characteristics such as polydispersity or complex protein solutions may affect analysis results. This study systematically addressed these key issues. The integrated syringe pump was used to increase the sample volume analyzed. It was observed that measurements recorded under flow caused a reduction in total particle counts for both polystyrene and protein particles compared to those collected under static conditions. In addition, data for polydisperse samples tended to lose peak resolution at higher flow rates, masking distinct particle populations. Furthermore, in a bimodal particle population, a bias was seen toward the larger species within the sample. The impacts of filtration on an agitated intravenous immunoglobulin sample and operating parameters including “MINexps” and “blur” were investigated to optimize the method. Taken together, this study provides recommendations on instrument settings and sample preparations to properly characterize complex samples.

Introduction

As nanotechnology is rapidly being applied to a wide range of research and manufacturing fields, the ability to accurately monitor and characterize nanoparticles (particles having a diameter smaller than 1 μm) is becoming increasingly important.[1] Many methods are currently available to characterize nanoparticles, including scanning electron microscopy, size exclusion chromatography, analytical ultracentrifugation, dynamic light scattering (DLS), and asymmetrical flow field-flow fractionation. However, these methods have limitations such as low throughput, limited quantitative data, high cost, and extensive data analysis and are best suited as a secondary method for characterization.[2-5] Of these methods, DLS has been used most widely because it provides relatively accurate sizing data for nanoparticles. However, problems inherent to the technique are that the presence of large aggregates introduces a bias toward larger particle sizes and particle concentration cannot be determined.[6] A recently emerged technique, nanoparticle tracking analysis (NTA), overcomes several of these limitations and is rapidly becoming a standard method for nanoparticle characterization.

With NTA analysis the sample is illuminated by an integrated laser, and a video of the nanoparticles scattering light in the solution is recorded through a microscope coupled to a high resolution camera. The NTA software uses the recorded videos to identify and track the spatial movement of every particle in the viewing frame in 2 dimensions. Using the Stokes-Einstein equation, the diffusion coefficient of individual nanoparticles is calculated from the video and converted into a hydrodynamic diameter to obtain a particle's size.[7]

This tracking method provides numerous advantages over methods such as DLS, including higher resolution for the particle distribution as it includes individual sizing and intensity data for every tracked particle rather than an ensemble measurement of all particles.[8] In addition, individual particle tracking allows for

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analysis of samples with a closer difference in sizes of particles.[8,9] Additionally, particle concentrations can be calculated because the illuminated field assessed during analysis can be estimated. This versatility over DLS has allowed NTA to be applied in a wide variety of research areas involving nanoparticles, including the following: (1) the characterization and quantification of nanoparticles for drug delivery and targeting[10-12]; (2) quality and stability assessments of therapeutic protein products[13-15]; (3) virus characterization[16]; (4) characterization of exosomes and microvesicles[17]; and (5) mechanistic studies of protein aggregation.[18]

Despite its wide use in several research areas, NTA has several limitations including the small sample volume analyzed and interference caused by sample turbidity.[19] Furthermore, NTA data acquisition and analysis remain prone to user input and bias on account of the high number of acquisition and analysis parameters available in the software, as well as the subjectivity in selecting focal depth of the microscope for video capture.[5,20] As such, calculations of particle concentrations and size distributions have been highly dependent on user-defined hardware and software settings for which the optimal choices must be empirically determined by the user.[16,20] For robust and reliable data collection, parameters that must be optimized by individual users include camera shutter and gain and analysis parameters such as blur and detection threshold. These parameters chosen by different users may vary, causing differences in reported results among users.[5] Furthermore, sample particle concentration and size ranges must fall within the upper and lower limits of the instrument.[19]

To reduce inter- and intra-day variation, expert training is recommended for accurate particle sizing and counting,[20] and currently attempts to standardize inter-laboratory data collection and analysis of nanoparticles are under way.[5] Recent studies with simple synthetic nanoparticle solutions have emphasized standardizing sample acquisition and analysis protocols that cover sample handling and storage, sample preparation (i.e., sample dilutions), video capture, data analysis, and improvements in statistical analysis, resulting in improvements to both sizing precision and reproducibility on identical samples analyzed between different laboratories.[5] Prior analyses by Chen et al. and Kramberger et al.[16] of system parameters and solution characteristics have been performed using older NTA software versions (version 2.2 and prior) to identify key acquisition and analysis parameters that affect particle characterization.[16,21] These are the camera settings of shutter and gain and the analysis parameters of blur, detection threshold, minimum expected particle size (MINexps), and complete tracking numbers. To help mitigate these issues, both studies offer practical recommendations to optimize these key settings with the older NTA software.

This study aims to further address these key issues in applying NTA (and newer NTA software versions) to obtain reliable and robust data when characterizing nanoparticles in more complex nanoparticle-containing solutions, including polydisperse samples. One general concern during NTA analysis is the low sample volume analyzed per viewing frame (approximately 4.0×10^{-8} mL). In order to obtain a particles/milliliter concentration, a multiplication factor of several million (approximately 1.5×10^{7}) is needed.[21] To increase the sample volume analyzed, the sample can be flowed through the system. Therefore, we examined the effects of flow rate on particle sizing, concentration, and polydispersity. In addition, on account of the ability to use scripting functions and the syringe pump for extensive analysis of a sample, time-dependent effects on the sizing and concentrations were explored. Furthermore, because we recommend using a flow rate for the sample,[21] the effects of key parameters (MINexps and blur) on particle concentration and characterization when flowing a sample were examined in detail. Unlike most prior analyses of particle characterization using NTA, this study used a newer software version in which several of these key analysis settings have been standardized, and thus these were also assessed in detail. Finally, because agitated protein samples may contain micron-sized and larger particles, the effect of filtration on a sample was examined. To address these issues, a systematic analysis using both synthetic and protein particles was performed using the NanoSight NS300 instrument with the integrated syringe pump.

Materials and Methods

Materials

The polyclonal therapeutic antibody product intravenous immunoglobulin (IVIg; Gammagard Liquid, Baxter HealthCare) was purchased from the University of Colorado at Boulder's Wardenburg Pharmacy (Boulder, CO) in liquid formulation at a 100 mg/mL concentration. For protein studies, IVIg was diluted to the indicated concentrations using formulation buffer (0.25 M glycine buffer, pH 4.61). The National Institute of Standards and Technology–traceable silica (8000 series) microspheres and polystyrene (PS) bead size and concentration standards (4000 series) were purchased from Thermo Scientific (Waltham, MA). PS bead concentration standards used a wt/vol estimation for determining bead concentration, as determined by the manufacturer. All chemicals were purchased from Fisher Scientific (Hampton, NH) and were of reagent grade or higher quality.

Instrument Configuration and Experimental Methods

A NanoSight Model NS300 (Malvern Instruments Ltd., Amesbury, UK) equipped with a 488-nm laser excitation source, high sensitivity scientific CMOS camera, and integrated syringe pump was used for this study. NTA 2.3 (software build 033) was used for data collection and analysis. Samples were first manually injected into the sample chamber using 1 mL silicon oil-free plastic syringes (National Scientific Company, Rockwood, TN), and then the syringe was placed in the syringe pump. All samples were prepared at 1 mL or greater volume for analysis. Analysis of all samples used the integrated syringe pump for data acquisition, regardless of flow rate indicated, including flow = 0 samples. Unless indicated, all samples used default Auto blur setting and automatic MINexps setting. All video capture and analysis settings, including camera shutter, camera gain, and detection threshold, remained identical for all samples in an individual experiment. All samples collected and analyzed had greater than 200 completed tracks for synthetic particles or 400 complete tracks for protein particles, as previously recommended.[16,21]

Instrument Cleaning Procedure

decon90 was obtained from Fisher Scientific (Waltham, MA) and diluted in MilliQ water to prepare a 1.5% solution. A 1-mL syringe containing diluted decon90 was placed in the syringe pump, and solution was flowed through the instrument tubing and sample chamber at a speed of 1000 (arbitrary value, instrument setting) until the solution was completely expelled from the syringe. Next, the instrument was manually washed with several milliliters of MilliQ water using a 10-mL plastic syringe (National Scientific Company). Upon completion, there were few to no particles present in the resulting sample window.

Flow Rate and Filtration Analysis

PS bead size standards were obtained with pre-calculated concentrations from the manufacturer (1×10^{9} particles/mL), which